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Central Antinociceptive Effects of *Cissus sicyoides* on Mice

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Abstract

The acute and chronic treatment of mice with a hydroalcohol extract from the leaves of *Cissus sicyoides* L. (Vitaceae) (CS) at doses of 300, 600, and 1000 mg/kg, kg, by intraperitoneal administration, produced a significant central antinociceptive effect on the hot plate, tail immersion, and acetic acid–induced writhing, tests, and the effect was inhibited by naloxone. CS was given to mice daily for 30 days at a dose of 600 mg/kg, causing an effect similar to that of drugs with typical action on opioid receptors. The effective dose (ED_{50}) was approximately 600 mg/kg in mice.

Keywords: Analgesic effect, *Cissus sicyoides*, CNS activity, morphine, naloxone.

Introduction

Medicinal plants have been used extensively by the Brazilian people to treat bodily ailments (Almeida, 1993). A case in point is *Cissus sicyoides* L. (Vitaceae) (CS) (Pepato et al., 2003), popularly known in Brazil as "insulinas, cipo-pucá, bejuco de porra, bejuco caro, pucí, anil trepador, and bejuco ubí" (Abreu et al., 2003). *Cissus sicyoides* is a plant originally from the Dominican Republic (Cano & Volpato, 2004). It is used in folk medicine as a diuretic, anti-inflammatory (Toledo, 1983) and antidiabetic (Garcia et al., 2000; Abreu et al., 2003; Pepato et al., 2003; Viana et al., 2004). It has demonstrated a vasoconstrictor effect on guinea-pig aorta rings (Garcia et al., 1997; Mundey et al., 2000) and antibacterial activity (Garcia et al., 1999). Studies with tea induced an increase in the amount of chromosomal damage in bone marrow cells without altering the cell division cycle (Vicentini et al., 2001). This plant also presents antibacterial and oxytoxic activities (Sáenz et al., 2000), and cytotoxic activity (Feng, 1964). CS also contains significant amounts of α-tocopherol, a compound proved to be a useful adjunct to anticonvulsant drugs in clinical studies (Barbosa, 1994). The phytochemical study showed flavonoids kaempferol 3-*O*-rhamnoside and quecetin 3-*O*-rhamnoside obtained from aerial plants of CS (Beltrame et al., 2001 & 2002). The genus *Cissus* contains sterols, quinones, and phenolic compounds in its leaves. It also contains anthocyanins, saponins, and flavonoids in its fruit (Toledo, 1983).

This study evaluates the possible central effects of a hydroalcohol extract from the leaves of CS in various behavioral tests. This study aimed to determine the possible central analgesic activity of CS using the hot plate, tail immersion, and acetic acid–induced writhing tests, and to determine its persistence, using a dose of 600 mg/kg (i.p.) of CS (Almeida et al., 2003) for 30 days.

Materials and Methods

Plant material

Aerial parts of CS were collected in the vicinity of Recife, State of Pernambuco, Brazil, in January 2004. The plant was identified by Prof. Marlene Carvalho de Alencar Barbosa of Universidade Federal de Pernambuco, and a voucher specimen was deposited at in the Geraldo Mariz herbarium (UFP) under no. 29040 of the Botanical Department of the Federal University of Pernambuco.

Preparation of the extract

The leaves were washed, dried at room temperature (approximately 28°C) in the laboratory for approximately

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25 days, and ground in a mill to a grain size of <1 mm. Then, 360 g of the powdered plant material were added to 1000 ml of alcohol and water (70:30). The dry leaf powder yielded 30% of extract. For pharmacological testing, the extract was dissolved in saline plus Tween 80 (0.025%).

Animals

Male and female Swiss albino mice with body weight 20–30 g, 2 months old at the beginning of the experiment, were used. The animals were housed in groups of six per cage, with a light/dark period of 12 h (6:00 AM to 6:00 PM). They were given food and water *ad libitum*. All experiments were conducted between 10:00 AM and 4:00 PM. Female mice were tested without monitoring the estrus cycle. All the animals were carefully monitored and maintained in accordance with the ethical recommendation of the Brazilian College of Animal Experimentation (COBEA) and the National Institute of Health Guide for Care and use of Laboratory Animals.

Drugs

Morphine and naloxone were obtained from Sigma (St. Louis, MO, USA). The extract was dissolved in 0.025% Tween 80 and diluted with distilled water. All solutions were administered by the intraperitoneal (i.p.) route in volume of 0.1 ml/10 g body weight.

Hot plate test

Mice were divided into five groups, each group of six animals. The tests followed the method described by Yeh and Mitchel (1971). The test animals received three doses (300, 600, and 1000 mg/kg, i.p.) of CS 1 h before measurements. In the control group, saline was administered i.p., and morphine (3 mg/kg, i.p.) was given to the standard group. Each assessment was carried out for a period of 15 min and the tests were performed at 30, 60, 90, and 120 min intervals after the respective treatments.

Tail immersion test

The mice were divided into five groups of six animals in each group. Group I received the vehicle (0.1 ml/10 g), and group II, which served as standard group, received morphine (6 mg/kg, i.p.). The analgesic activity was evaluated 1 h after administration of the extract, and morphine was evaluated 15 min after administration. The tail (up to 5 cm) was then dipped into a pot of water maintained at 55°C. The time (in seconds) the mouse took to withdraw its tail clearly out of the water was taken as the reaction time according to Janssen et al. (1963).

Acetic acid–induced writhing test

The analgesic effect of extract was evaluated in mice using the acetic acid–induced writhing test. Mice were divided into six groups of six animals each. Vehicle (controls), extract (300, 600, and 1000 mg/kg, i.p.) and morphine (3 mg/kg, i.p.) were administered. Sixty minutes after treatment, acetic acid (0.85% v/v solution) was injected i.p. at a dose of 10 mg/kg. The number of writings was counted and recorded for 15 min (Almeida et al., 2000).

Antagonism of the antinociceptive effect of CS by pretreatment with naloxone

In the experiments, six groups of six animals in each group were used. All the animals had naloxone administered at dose of 2 mg/kg (i.p.). After 15 min, the test groups were give doses of 300, 600, and 1000 mg/kg (i.p.) of CS. The control group received saline (0.1 ml/10 g, i.p.) and the standard group had morphine (3 mg/kg, i.p.) administered. The assessments were conducted at 30, 60, 90, and 120 min in accordance with the method proposed by Younos et al. (1990).

Effect of CS and morphine administered for 30 days determined by tail immersion test

In these experiments, three groups of six animals in each group were used to study the chronic administration of CS (600 mg/kg, i.p.) and morphine (3 mg/kg, i.p.) The control group received saline (0.1 ml/10 g, i.p.). Analgesia was assessed by the tail immersion test according to Janssen et al. (1963) and Almeida et al. (2003) before the start of treatment (for baseline values) and on days 7, 14, 21, and 30 after administration of drugs and saline.

Statistical analysis

The data was submitted to analysis of variance (ANOVA). Post hoc comparison between individual treatments and controls was made using Dunnett's multiple comparison tests or Student's *t*-test, depending on the case. The results obtained were considered significant when $p < 0.05$.

Results

Effect of CS in the hot plate test

As shown in Table 1, the hydroalcoholic extract of CS, administered intraperitoneally at doses up to 1000 mg/kg, produced a significant antinociceptive action when compared with the control group and one similar to that of morphine at 3 mg/kg (i.p.). This action of CS was confirmed by the blocking effect of naloxone.

Table 1. Effect of *C. sicyoides* (CS) and naloxone on mice assessed by the hot plate test.

Treatment mg/kg (i.p.)	Reaction time (s), mean ± SD			
	Basal			
	30 min	60 min	90 min	120 min
Saline (−) 6.4 ± 1.5	5.8 ± 0.9	5.1 ± 1.1	7.1 ± 0.7	5.9 ± 1.2
CS (300) 41.4 ± 1.3*	6.8 ± 1.4	9.3 ± 0.6*	20.7 ± 1.2*	26.4 ± 2.2*
CS (600) 21.0 ± 1.0*	6.5 ± 1.5	16.4 ± 1.5*	18.1 ± 1.4*	21.7 ± 1.0*
CS (1000) 24.2 ± 1.8*	7.2 ± 1.5	20.9 ± 1.6*	19.2 ± 0.9*	19.6 ± 1.3*
CS (300) + Naloxone (2) 7.3 ± 0.8	6.2 ± 0.8	5.9 ± 03	7.1 ± 0.4	7.1 ± 0.7
CS (600) + naloxone (2) 7.6 ± 0.6	6.8 ± 1.4	6.7 ± 0.5	7.1 ± 0.5	7.0 ± 0.6
CS (1000) + naloxone (2) 7.2 ± 0.6	6.9 ± 1.4	7.3 ± 0.5	7.3 ± 0.7	7.7 ± 0.5
Morphine (3) + naloxone (2) 7.3 + 0.9	6.7 + 1.4	7.5 + 0.4	7.5 + 0.7	7.5 + 0.5

n = 6. *p < 0.01 compared with saline group values.

Table 2. Effect of *C. sicyoides* (CS) on mice assessed by the tail immersion test.

Treatment mg/kg (i.p.)	Reaction time (s), mean ± SD			
	Basal			
	30 min	60 min	90 min	120 min
Saline (−) 6.4 ± 1.5	5.5 ± 0.7	5.1 ± 1.1	6.1 ± 0.7	6.1 ± 0.7
CS (300) 8.8 ± 0.6*	6.3 ± 0.3	7.1 ± 0.9	8.2 ± 0.4*	12.7 ± 0.9**
CS (600) 8.4 ± 0.6*	6.1 ± 0.4	8.5 ± 1.5	13.8 ± 0.8**	13.5 ± 0.7**
CS (1000) 8.2 ± 0.9*	7.3 ± 0.4	20.9 ± 1.6	40.4 ± 0.3**	42.1 ± 0.3**
CS (300) + naloxone (2) 7.3 ± 0.8	7.1 ± 0.9	5.9 ± 03	7.1 ± 0.4	7.1 ± 0.7
CS (600) + naloxone (2) 7.6 ± 0.6	6.5 ± 0.6	6.7 ± 0.5	7.1 ± 0.5	7.0 ± 0.6
CS (1000) + naloxone (2) 7.2 ± 0.6	6.9 ± 1.0	7.3 ± 0.5	7.3 ± 0.7	7.7 ± 0.5
Morphine (3) + naloxone (2) 6.2 ± 0.7	6.7 ± 1.4	7.1 ± 0.9	6.4 ± 0.6	6.0 ± 0.7

n = 6. *p < 0.05 compared with saline group values.
**p < 0.01 compared with saline group values.

Effect of CS in the tail immersion test

The CS (Table 2) administered intraperitoneally at doses up to 1000 mg/kg produced a significant antinociceptive action when compared with the control group and one similar to the action of morphine (3 mg/kg, i.p.). This action was the blocking effect of naloxone.

Acetic acid–induced writhing in mice

The CS produced a near maximal inhibition of the writhing response similar to that of morphine (Table 3).

Table 3. Effect of *C. sicyoides* (CS) evaluated using acetic acid–induced writhing in mice.

Treatment (mg/kg, i.p.)	Writhing (mean ± SD)
Saline (control)	42.1 ± 4.8
CS (300)	15.8 ± 5.9*
CS (600)	6.1 ± 1.4*
CS (1000)	8.3 ± 1.3*
Morphine (3)	5.7 ± 0.4*

n = 6. *p < 0.01 compared with saline group values.

Effect of CS and morphine administered for 30 days determined by the tail immersion test in mice

The results of this test are summarized in Table 4.

Table 4. Effect of *C. sicyoides* (CS) administration for period of 30 days on mice assessed by tail immersion test.

Treatment mg/kg (i.p.)	Reaction time (s), mean ± SD				
	Basal				
		7th day	14th day	21th day	30th day
Saline (−)	5.8 ± 0.9	5.1 ± 1.1	5.1 ± 0.7	5.9 ± 1.2	6.4 ± 1.5
CS (600)	6.5 ± 1.5	16.4 ± 1.5*	6.7 ± 1.4	5.7 ± 1.0	5.1 ± 1.0
Morphine (6)	6.7 + 1.4	17.5 + 1.4*	7.1 + 2.7	7.5 + 0.5	5.9 + 0.9

n = 6. *p < 0.05 compared with saline group values.

Discussion

Medicinal plants are widely used in Brazil and have been a substantial source of ethnopharmacological information for the identification of phytochemical substances with therapeutic potential, as in the case of leaves of *Cissus sicyoides* used in diuretic, anti-inflammatory (Garcia, 2000), and antidiabetic (Beltrame, 2001; Pepato et al., 2003) treatments. The analgesic action presented

by CS involves supraspinal as well as spinal components as demonstrated by the use of the hot plate (Yaksh & Rubi, 1999) and tail immersion tests (Mayer & Liebeskind, 1974), respectively. The results suggest that CS has a central analgesic effect, as evidenced by increase in reaction time of mice in the hot plate test and tail immersion test. The central analgesic action was confirmed by the blocking effect of naloxone, a specific morphinomimetic receptor antagonist (Belvisi et al., 1998; Quock et al., 1999; Mundey et al., 2000) and shows classic tolerance, characteristic of morphine and other opioid substances. This antinociceptive effect of CS may be related to the reduction in Ca^{2+} influx at the axon terminal of the afferent nerve inducing a decrease in adenylyl cycle activities, which results in decreased levels of cyclic AMP and efflux of K^+ ions. The latter lead to hyperpolarization of the nerve and finally to an apparent antinociceptive effect (Dickinson & Fleetwood-Walker, 1998; Grubb, 1998; Yaksh & Rubi, 1999). Moreover, CS shows a tolerance effect, characteristic of morphine and other opioid substances.

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